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Supporting Information

Streptocollin, a Type IV Lanthipeptide Produced by *Streptomyces collinus* Tü 365

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Content

Media and culture conditions

E. coli NovaBlue (Novagen) used as general cloning strain was grown in Luria broth (Sambrook, 2001), supplemented with 100 µg/mL apramycin to maintain plasmids. Intergeneric conjugation (Wohlleben, et al., 1994) was performed using a methylation deficient strain ET12567 (MacNeil, et al., 1992) containing the mobilizing RP4 derivative PUB307 (Bennett, et al., 1977) cultivated in Luria broth, supplemented with 50 µg/mL kanamycin, 25 µg/mL chloramphenicol and 50 µg/mL apramycin to maintain plasmids/cosmids. For starter cultures or genomic DNA isolation *S. collinus* Tü 365 and *S. coelicolor* strains were grown in TSB medium (Tryptic Soy Broth, Becton, Dickinson and company) at 29 °C, 180 rpm in an Erlenmeyer flask with one baffle and a steel coil. Producing strains were cultivated on Petri dishes containing 30 mL of SFM-agar (20 g mannitol, 20 g soy meal full fat, 16 g agar, 1 L tap water). Liquid/solid media were supplemented with 50 µg/mL apramycin to select for strains carrying integrated antibiotic resistance genes.

General protocols

Genomic DNA from *Streptomyces* strains was isolated using NucleoSpin® Tissue Columns from Macherey-Nagel according to the manufacturer's protocol.

DNA purification was performed with GE Healthcare illustraTM plasmidPrep Mini Spin Kit and GE Healthcare illustraTM GFXTM PCR DNA and Gel Band Purification Kit, respectively. For the amplification of DNA-fragments by PCR, ProofStartTM DNA

Polymerase (QIAGEN) was used. DNA was digested with restriction enzymes from MBI Fermentas according to the producer's description.

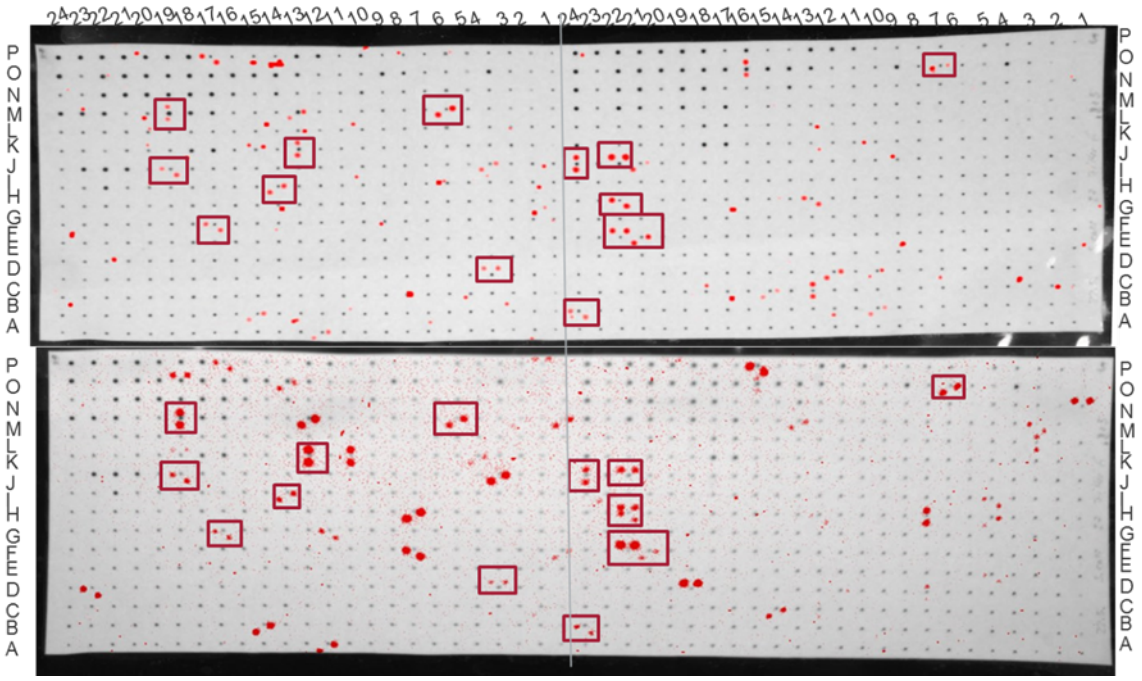


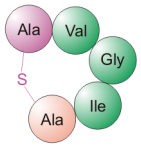
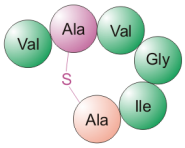
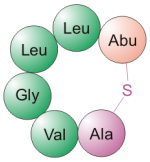
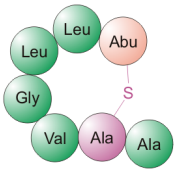
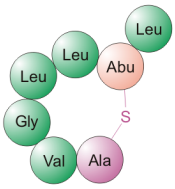
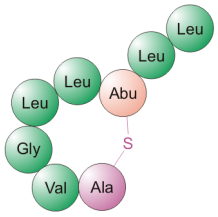
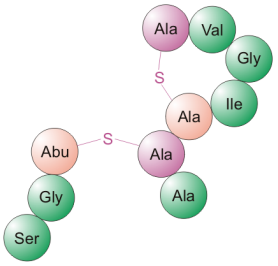
Figure S1. Cosmid library screening. Identification of cosmid clones encoding the lanthipeptide biosynthesis gene cluster from *S. collinus* Tü 365. Hybridization results received through hybridization with two labeled DNA probes generated for the detection of the left and right border of the lanthipeptide cluster are shown in red. Cosmid clones marked in red boxes indicate the detection of clones with both probes. 14 cosmids containing the entire lanthipeptide biosynthesis cluster were identified.

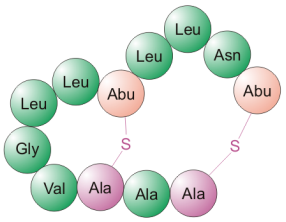
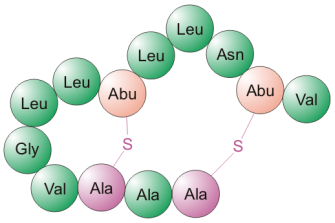
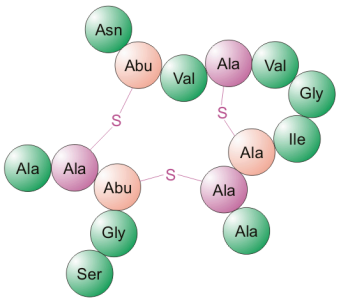
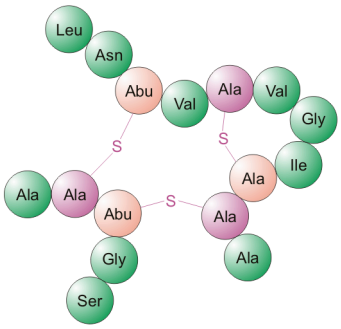
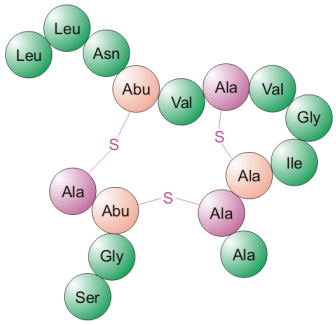
Table S1: Strains and plasmids used in this study

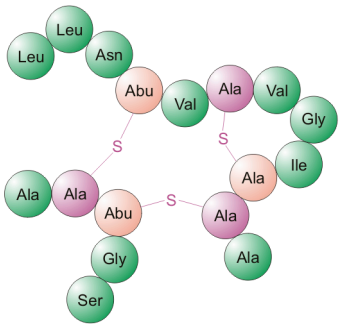
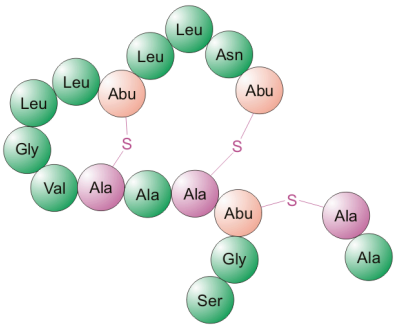
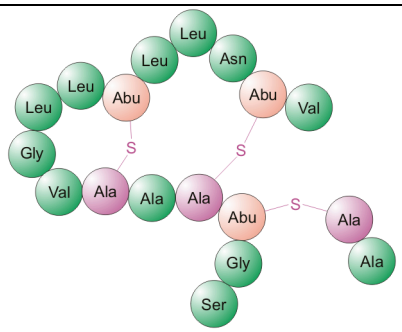
Strain/ Plasmid/ Cosmid	Description	Source
Plasmids		
pRM4.2	pSET152*erm*E*p-derivate (ΦC31 integration vector with *ermE**p promoter, Apra^{R}) with artificial RBS, used for construction of pDIpro	(Menges, *et al.,* 2007)
pDIpro	pRM4 derivate with the *stcL* gene fragment, without ΦC31 integrase gene and attachment site *attP*; used for integration of *ermE**p in *S. collinus* Tü 365	This study
pUB307	self-transmissible plasmid that mobilizes other plasmids in trans for DNA transfer into hosts: RP4, neo	(Bennett, *et al.,* 1977)
Cosmid		
pOJ436	3 *cos* sites, *aac*(3)IV, ΦC31 integration system	(Bierman, *et al.*, 1992)
Strains		
***E. coli* NovaBlue**	*recA*1, *endA*1, *gyrA*96, *thi*-1, *hsdR*17 (rK12^{-},mK12^{+}) *supE*44, *relA*1, *lac* [F', *pro*AB, *lacI*^{q}, *lacZ*ΔM15, Tn*10*] (Tet^{R})	Novagen
***E. coli* ET12567**	DNA methylation deficient donor strain for conjugation: F2 *dam*13::Tn*9, dcm*-6, *hsdM, hsdR, recF143, zjj-202::*Tn*10, galK2, galT22 ara-14, lacY1,xyl-5, leuB6, thi-1, tonA31, rpsL136, hisG4, tsx-78, mtl-1, glnV44*	(MacNeil, *et al.,* 1992)
***S. collinus* Tü 365**	*S. collinus* wild type strain Tü 365, kirromycin producer	(Wolf and Zähner, 1972)

Strain/ Plasmid/ Cosmid	Description	Source
***S. collinus* Tü 365-pDI1**	*S. collinus* Tü 365 with the plasmid pDIpro integrated into the chromosome through homologous recombination, $Apra^R$	This study
***S. coelicolor* M1146**	host strain for heterologous expression derived from *S. coelicolor* M145: *Δact, Δred, Δcpk, Δcda*	(Gomez-Escribano and Bibb, 2011)
***S. coelicolor* M1152**	host strain for heterologous expression derived from *S. coelicolor* M145: *Δact, Δred, Δcpk, Δcda, rpoB[C1298T]*	(Gomez-Escribano and Bibb, 2011)
M1146 + *stc* cluster	M1146 with integrated cosmid harboring the *stc* cluster	This study
M1152 + *stc* cluster	M1152 with integrated cosmid harboring the *stc* cluster	This study

Table S2: ppm values of streptocollin fragments.

Fragment	Calculated	Experimental	ppm	According structure
$[y_7\text{-}b_{21}]$	442.21242	442.21228	-0.32	
$[y_8\text{-}b_{21}]$	541.28083	541.28072	-0.20	
$[y_{18}\text{-}b_{11}]$	569.31213	569.31190	-0.40	
$[y_{19}\text{-}b_{11}]$	640.34925	640.34960	-0.55	
$[y_{18}\text{-}b_{12}]$	682.3962	682.39618	-0.03	
$[y_{18}\text{-}b_{13}]$	795.48026	795.47980	-0.58	
$[y_{20}\text{-}b_{16}]$*	861.35989	861.36041	0.60	

[y_{20}-b_{15}]	1166.60660	1166.60608	-0.45	
[y_{20}-b_{16}]	1265.67502	1265.67369	-1.05	
[y_{18}-b_{13}]*	1331.55464	1331.55414	-0.38	
[y_{18}-b_{12}]*	1444.63871	1444.63940	0.48	
[y_{19}-b_{11}]*	1486.68566	1486.68600	0.23	

$[y_{18}\text{-}b_{11}]^*$	1557.72277	1557.72182	-0.61	
$[y_8\text{-}b_{21}]^*$	1585.75407	1585.75293	-0.72	
$[y_7\text{-}b_{21}]^*$	1684.82248	1684.82232	-0.09	

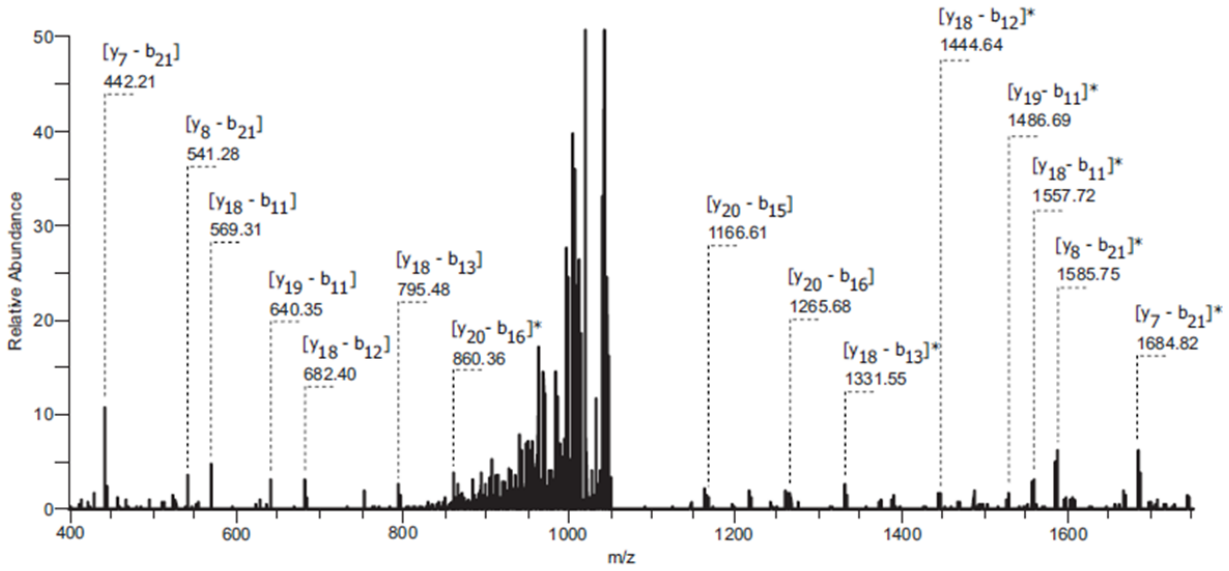


Figure S2. Full-size spectrum of streptocollin fragmentation using collision-induced dissociation, CE = 40.

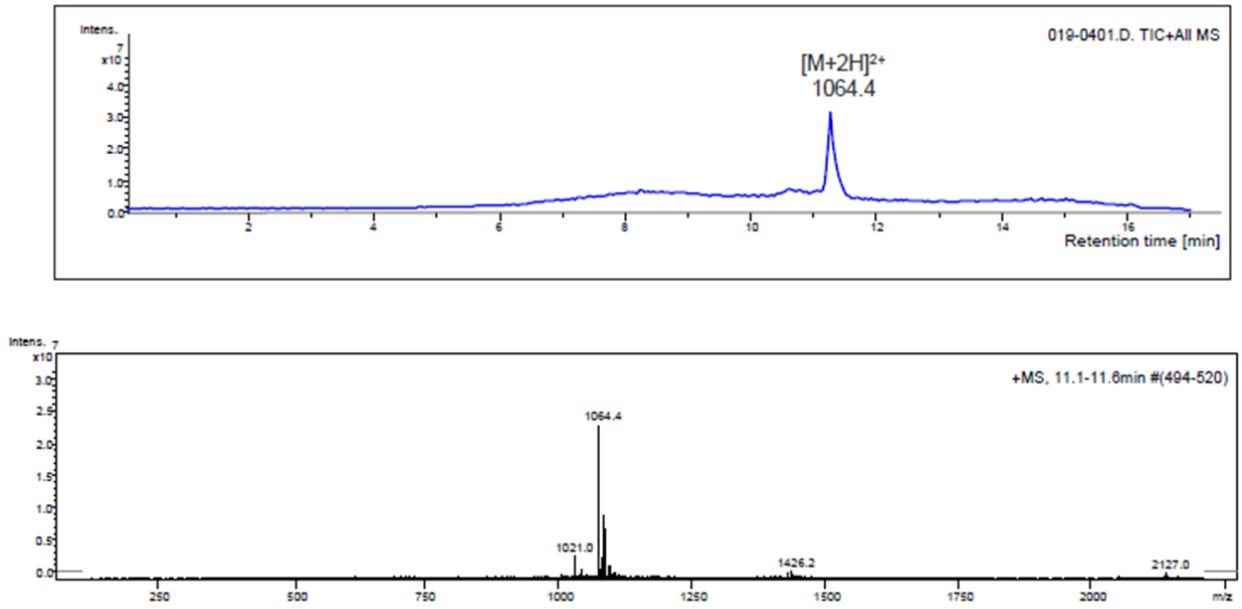


Figure S3. TIC-MS (total ion chromatogram) of streptocollin after isolation from recombinant strain *S. coelicolor* M1146.

Table S3. Growth conditions for indicator strains used in antimicrobial assays

Strain	**Medium**	**Temperature**
E. coli K12	KM1(bacto nutrient broth 8 g/L, NaCl 5 g/L, pH7.2)	37°C
B. subtilis	DSMZ (bacto peptone 5g/L, malt extract 3g/L, $MnSO_4$ x H_2O 10mg/L, pH7)	
M. luteus	KM1	37°C
Streptomyces strains: *S. albus, S. violaceoruber, S. pristinaespiralis, S. viridochromogenes, S. coelicolor M145, S. virginae, S. collinus Tü 365*	TSB (tryptic soy broth)	29°C
Saccharomyces cerevisiae	KM5 (yeast extract 4g/L, malt extract 10g/L, glucose 4g/L pH5.5)	29°C

References

Bennett, P.M., Grinsted, J., and Richmond, M.H. (1977). Transposition of TnA does not generate deletions. Mol Gen Genet 154, 205-211.

Bierman, M., Logan, R., O'Brien, K., Seno, E.T., Rao, R.N., and Schoner, B.E. (1992). Plasmid cloning vectors for the conjugal transfer of DNA from Escherichia coli to Streptomyces spp. Gene 116, 43-49.

Gomez-Escribano, J.P., and Bibb, M.J. (2011). Engineering Streptomyces coelicolor for heterologous expression of secondary metabolite gene clusters. Microb Biotechnol 4, 207-215.

MacNeil, D.J., Gewain, K.M., Ruby, C.L., Dezeny, G., Gibbons, P.H., and MacNeil, T. (1992). Analysis of Streptomyces avermitilis genes required for avermectin biosynthesis utilizing a novel integration vector. Gene 111, 61-68.

Menges, R., Muth, G., Wohlleben, W., and Stegmann, E. (2007). The ABC transporter Tba of Amycolatopsis balhimycina is required for efficient export of the glycopeptide antibiotic balhimycin. Appl Microbiol Biotechnol 77, 125-134.

Sambrook, J. (2001). Molecular cloning: a laboratory manual (Cold Spring Harbor, N.Y. : Cold Spring Harbor Laboratory, c2001).

Wohlleben, W., Hartmann, V., Hillemann, D., Krey, K., Muth, G., Nussbaumer, B., and Pelzer, S. (1994). Transfer and establishment of DNA in Streptomyces (a brief review). Acta Microbiol Immunol Hung 41, 381-389.

Wolf, H., and Zähner, H. (1972). Metabolic products of microorganisms. 99. Kirromycin. Arch Mikrobiol 83, 147-154.